Sexual Differentiation Inside and Out

Introduction

The default phenotype is female. These words will be said many times. This lesson is not about the female phenotype, but rather the mechanisms by which a male genome (46,XY) transforms the default female phenotype to a male phenotype.

The *SRY* gene on the Y chromosome initiates the cascade of events. When everything goes the way it is supposed to, all phenotypic features that would have developed into the female phenotype develop into the male phenotype instead. We're going to state (without going into much detail) what the default female phenotype is, but we spend the majority of this lesson focusing on the male phenotype. "Phenotypic features" can be categorized into three areas: gonads and their cell types, tubes, and external genitalia. This introduction is also the summary, so don't worry if this comes at you too fast. The gonads can be ovaries (female phenotype) or testes (male phenotype). The cell types are defined by the gonad: the female gonad contains oogonia, granulosa cells, and theca cells, and the male gonad contains spermatogonia, Sertoli cells, and Leydig cells. The tubes make things a little complicated because there isn't simply a male phenotype and a female phenotype. The tubes (accurately called internal genitalia, but we use "tubes" so as not to invite confusion of internal with external genitalia) are derived from the Müllerian ducts and the mesonephric ducts. The female phenotype arises from the persistence of the Müllerian ducts and involution of the mesonephric ducts, whereas the male phenotype arises from the persistence of the mesonephric ducts and involution of the Müllerian ducts. But these are independent events, so the Müllerian ducts can either persist or involute, and separately, the mesonephric ducts can persist or involute. Finally, the external genitalia can have either the female phenotype (labia majora, labia minora, lower two-thirds of the vagina, and clitoris) or the male phenotype (scrotum, penis, and glans penis). This lesson is about sexual differentiation and the molecular mechanisms that facilitate that differentiation.

We end with some defects in the process. Because *the default phenotype is female,* the only disorder of female development is androgen excess (as in congenital adrenal hyperplasia). So, most of the female phenotype problems occur at puberty (where the default is neither male nor female), and are covered in Female Reproduction #6: *Reproductive Endocrinology: Puberty, Menopause, and the HPO*. The majority of disorders covered in this lesson those of genetic males with some or all of the female phenotype features.

Coelom is the most common way the word is written. It's use has started to see a downward trend and the use of celom is trending up. Still, coelom is vastly more popular. We use the word a lot in this lesson, so are calling it out on purpose. It is pronounced *SEE-lome* (like saying the letter c, then *lome* as in the lobe of a lung with a long o). The nomenclature gets unwieldly quickly but we'll be taking some nomenclature shortcuts to help you along.

Early Embryogenesis to Master Normal Differentiation

We're going to start with the hard part, because it comes first in the development of the gonads: early embryogenesis, week 4. Roll with this paragraph and don't scrutinize too hard. At this point, the nephrogenic cord has given rise to the mesonephros (the pronephros has already been involuted), the yolk sac is within the umbilical cord, and the first Body Cavity, the intraembryonic coelom, has formed. The mesonephros serves the purpose of filtration as the embryo develops kidneys. The yolk sac is the source of endodermal cells, connected to the embryo by the ventral (anterior) mesentery. The intraembryonic coelom is the original Body Cavity and will eventually become all the Body Cavities (pericardial cavity, pleural cavities, tunica vaginalis, peritoneal cavity). The mesentery is made of two sheets of the peritoneal cavity coming really close together. Body Cavities are fluid-filled sacs lined with mesothelium. All the facts! All the complicated words! Why does all of this matter? Because explaining the earliest changes correctly makes everything fall into place. Did you see it in the last lesson? Yes, you

just don't realize it. So here we go, saying this paragraph in many paragraphs to make things make sense. Look at Figure 2.1 as you read along with the text.

The mesonephros is derived from **mesodermal mesenchyme** ("meso" just means "middle"—there is no association between the middle kidney thing (mesonephros) and the middle embryonic-layer thing (mesoderm)). The earliest sign of the primordial gonad arises from the medial aspect of the mesonephros as a result of **mesodermal proliferation**. This proliferation forms a little bump on the medial aspect of the mesonephros termed the **genital ridge**. Together, the mesonephros and genital ridge make up the urogenital ridge (in the middle of the length of the embryo, not to be confused with the urogenital sinus at the tail end).

The **primary sex cords** form via the **proliferation of the mesothelium** of the intraembryonic coelom into the genital ridge, from cortex to medulla. The term "sex cord" doesn't help define what it is, but it is the name this thing has, and the sex cords generate the pathologic "sex cord-stromal tumors" we talk about later in this module. What matters more is where the primordial germ cells go and what gets expressed by their genetic makeup.

The **primordial germ cells** (PGCs) migrate from the yolk sac through the ventral mesentery and take up residence in the medulla and cortex of the genital ridge. The genetics of the PGCs (XY or XX) will determine what happens from here.

If the PGCs have the XX genotype, the medulla of the gonad involutes and is absorbed, and the cortical PGCs differentiate into dividing oogonia. By week 8, there are 600,000 oogonia, and by week 20, there are 6 million. Many oogonia die and are resorbed; the remainder become surrounded by a single layer of granulosa cells to form primordial follicles and enter meiosis to become **primary oocytes** (see Female Reproduction #1: *The Healthy Ovary*).

If the PGCs have the XY genotype, the cortex involutes and the medulla enlarges. The PGCs in the cortex (those that would have become oogonia) involute, while the PGCs of the medulla (those that will become spermatogonia) proliferate. But before they become the spermatogonia, the PGCs induce the mesothelial cells of the sex cords to become Sertoli cells. In turn, the Sertoli cells induce the nearby mesenchyme to become Leydig cells.

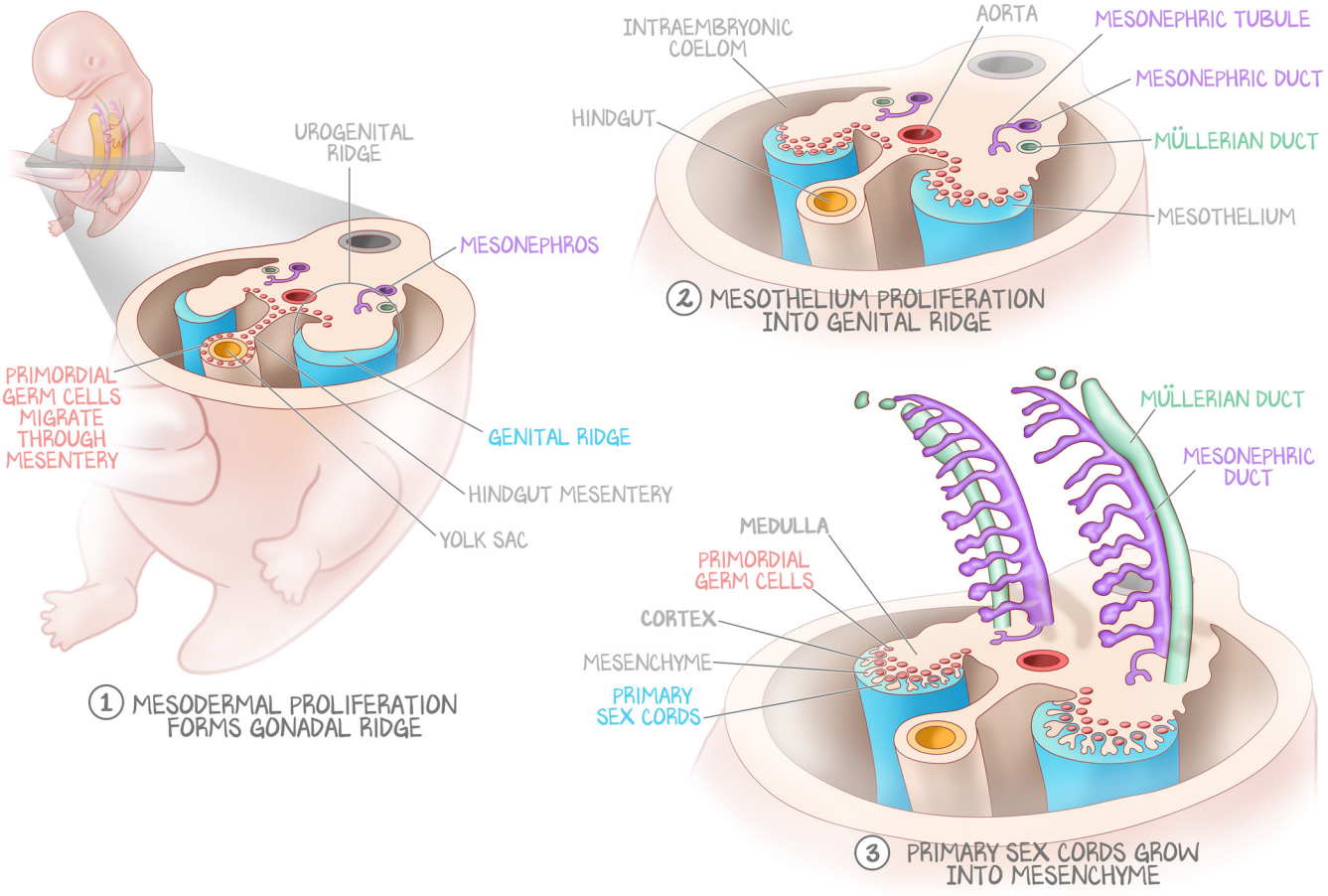

Figure 2.1: Early Embryogenesis of the Indifferent Gonad
(1) Mesoderm proliferates outward, forming the genital ridge. (2) Mesothelium, the lining of the intraembryonic coelom, proliferates into the genital ridge, while the endoderm-derived PGCs migrate into the genital ridge. (3) The mesoderm becomes the stroma, the mesothelium becomes the primary sex cords, and the PGCs populate the primary sex cords. Eventually, the gonads are polarized into the cortex (the outside, nearest the intraembryonic coelom) and medulla (near the hilum, where the mesonephric duct and blood vessels enter the gonad).

Now let's look at the mechanisms that make these things happen.

Gonads Are Determined by the *SRY* Gene

Now armed with perspective, let's go back to before there was any XX or XY phenotypical change to the gonads and start talking molecular details. The gonads are located in the genital ridge, the medial mesonephros. By week 4, the gonads can become both testes and ovaries, so they are termed indifferent gonads. The **indifferent gonad** is composed of an outer cortex and an inner medulla. In embryos with the male genotype (46,XY), the medulla differentiates into a testis, and the cortex regresses. Conversely, in embryos with the female genotype (46,XX), the cortex develops into an ovary, and the medulla regresses. Genotypic sex determines gonadal sex.

As we will say several times in this lesson, "*the default phenotype is the female phenotype.*" In the case of the indifferent gonads, that is only half true. We want you to learn that **no signal is required** for the gonad to become an **ovary** (*the default phenotype is the female phenotype*). However, in patients with Turner syndrome, wherein the patient lacks a second X chromosome (45,XO), the ovaries form as streaks, not full ovaries. Two X chromosomes (46,XX) are required for the indifferent gonads to become fully mature ovaries. In addition, patients with Klinefelter syndrome (47,XXY) develop testes. Those two are

the conditions to know the most about, as we will see them again when we discuss puberty and consider secondary sex characteristics. As a reinforcement of the material, at the end of this lesson, we'll discuss some examples of rare genetic events, such as translocation of a segment of the Y chromosome that encodes the signal to develop testes combined with another gene's promoter on a different chromosome (i.e., no Y chromosome, but a Y gene). But, although it is really the presence or absence of the *SRY* gene that determines gonadal differentiation (next paragraph), we want you to learn it as:

The presence of a Y chromosome differentiates the gonad into a testis; the absence of a Y chromosome differentiates the gonad into an ovary.

Without a specific signal from the Y chromosome, the ovary is the default (*the default phenotype is the female phenotype*). The specific signal is a transcription factor called **testis-determining factor** (**TDF**), encoded by the ***SRY* gene** found on the Y chromosome. It is evolutionarily ancient, has no introns, and is extremely well conserved. TDF isn't a cytokine or a receptor ligand, but rather an intracellular protein—every PGC either expresses TDF, or it doesn't. Without TDF expression, the cells of the indifferent gonad will become ovary cells (oogonia, granulosa, and theca cells); with TDF expression, they become testis cells (spermatogonia, Sertoli, and Leydig cells). The PGCs in the cortex express *Wnt4* and *Rspo1*, whose expression is reduced by TDF. The PGCs in the medulla express *Sox9* and *Fgf9*, whose expression is increased by TDF, thus enabling the cortex to involute and the medulla to proliferate.

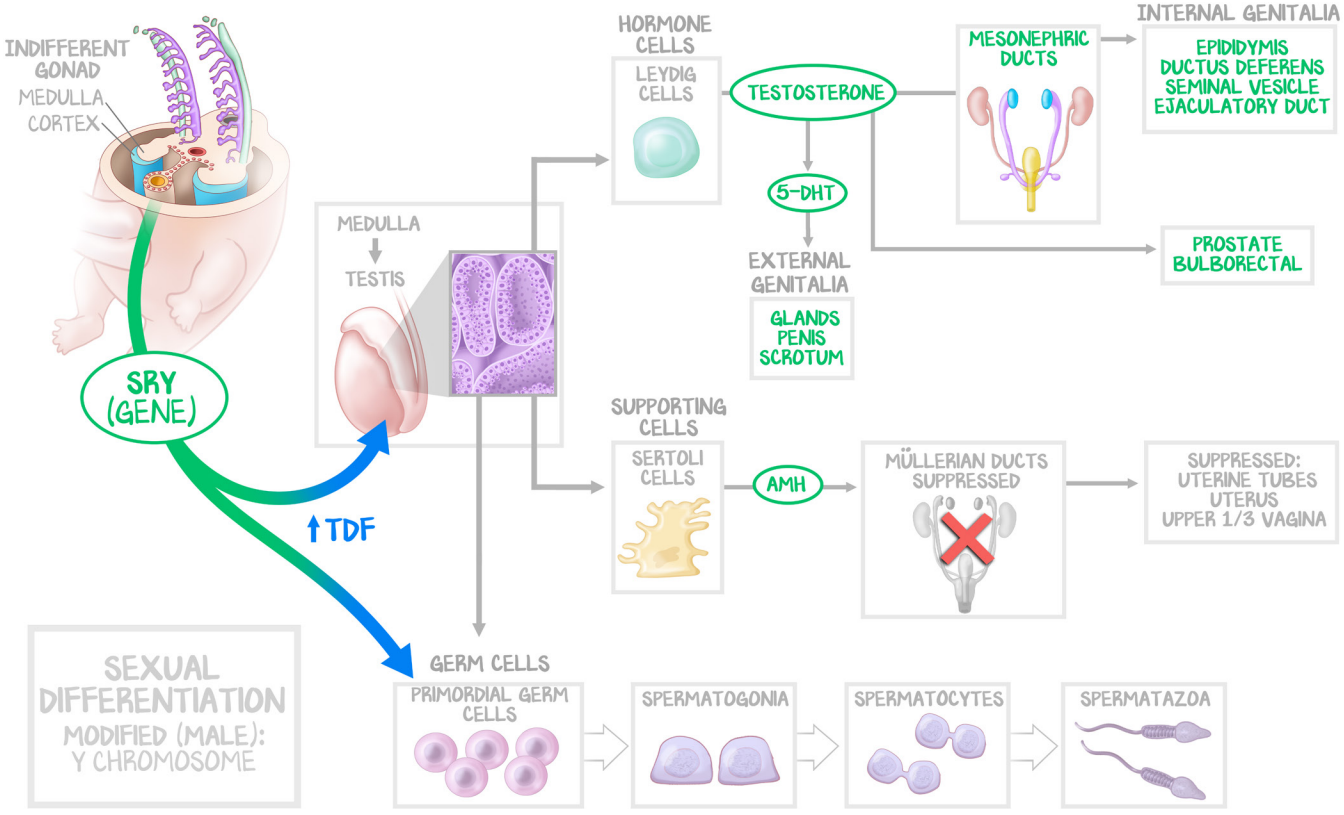

Figure 2.2: Differentiation of the Indifferent Gonad
In both sexes, the PGCs arrive from the yolk sac. TDF is produced via the *SRY* gene on the Y chromosome. If TDF is produced by the PGCs, the sex cords form and hollow out, and Sertoli cells are made. The tunica albuginea separates the seminiferous tubules from the coelom and mesothelium from which they are derived. Sertoli cells then induce the formation of Leydig cells. *The default phenotype is the female phenotype.* Granulosa cells are the nurturers of the germ cells (i.e., granulosa cells are essentially female Sertoli cells), theca cells are the androgen-producing interstitial cells (i.e., female Leydig cells), and germ cells are oogonia (i.e., female spermatogonia).

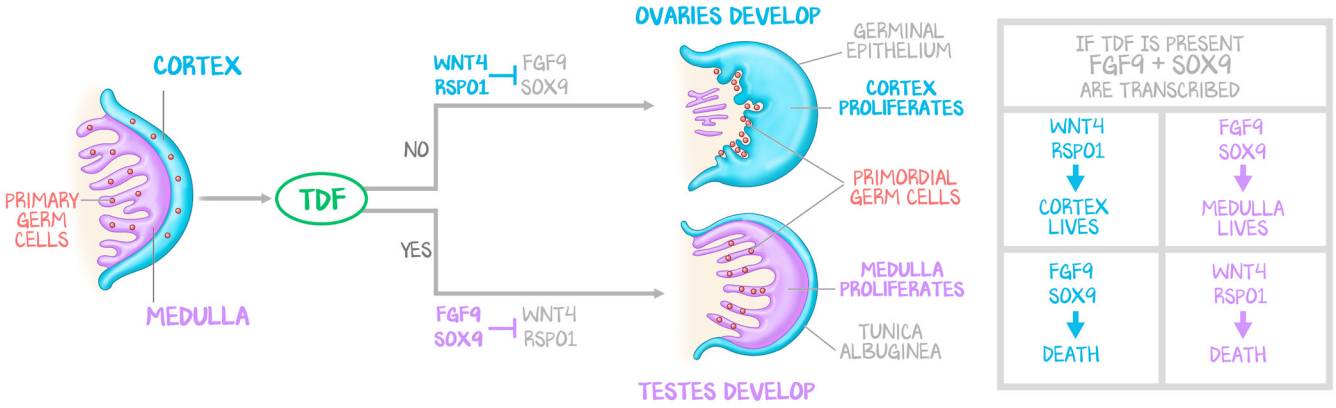

Figure 2.3: Differentiation of the Indifferent Gonad 2
Germ cells populate the cortex and medulla. Different proteins are transcribed based on the inherent genetics. In the cortex, WNT4 and RSPO1 mean survival, whereas FGF9 and SOX9 mean death. In the medulla, it is reversed—WNT4 and RSPO1 mean death, whereas FGF9 and SOX9 mean survival. In addition, WNT4 and RSPO1 inhibit the transcription of FGF9 and SOX9, whereas FGF9 and SOX9 inhibit the transcription of WNT4 and RSPO1. The default is for the cortex to live, the medulla to die, and the gonad to become the ovary. If the germ cells possess a Y chromosome, the SRY gene encodes the transcription factor called testis determining factor (TDF), which increases expression of FGF9 and SOX9, thereby inhibiting WNT4 and RSPO1 in both the cortex and medulla. In that case, the cortex dies, the medulla lives, and the gonad becomes a testis.

The germ cells of the ovary are the oogonia, whereas the PGCs of the testis are spermatogonia. The oogonia become primary oocytes before the end of embryogenesis, existing in the postnatal female only as oocytes. The spermatogonia will produce spermatocytes continuously throughout a male's life, from puberty until death. The cells that support the germ cells of the ovary (oocytes) are the granulosa cells, whereas the cells that support the germ cells of the testis (spermatogonia and spermatocytes) are the Sertoli cells. The interstitial cells that secrete lipophilic, cholesterol-based hormones (androgens) in the ovary are theca cells, whereas the hormone (testosterone)-producing interstitial cells of the testis are Leydig cells.

The order in which these cells are derived from a pluripotent genital ridge of both mesothelium and mesodermal mesenchyme is important. In male embryos, the PGCs that are to become follicles in the cortex involute, whereas the PGCs that are to become spermatogonia proliferate and overtake the involuting cortex. Those PGCs are not yet spermatogonia—they first must induce the mesothelium-derived sex cords to become Sertoli cells. After ensuring that their support system (Sertoli cells) is in place, the PGCs (under the direction of TDF) differentiate into spermatogonia. Together, the Sertoli cells and the spermatogonia will become the seminiferous tubules. But first, the Sertoli cells induce Leydig cell precursors to become Leydig cells. Leydig cells seem to arise from multiple tissues (both mesothelium and mesodermal mesenchyme). Regardless, the order of differentiation is PGC, then Sertoli, then Leydig.

Internal Genitalia Are Determined by Sertoli and Leydig Cells

Most people hear "genitalia" and think only of the external organs we can see. **Primary sex characteristics** are the structures that transport gametes, comprising both the internal and external genitalia. Internal genitalia is what we mean by "tubes" in our advanced organizer—gonads, tubes, external genitalia; we handle each of the three separately. In the last lesson, you learned that "tubes" comes down to the mesonephric duct (male phenotype) and Müllerian duct (female phenotype). There are two sets of tubes and, therefore, two signals that either support them or cause them to involute. As an aside, secondary sex characteristics have to do with sex behaviors, such as the development of pubic and axillary hair (a sign that the organism is approaching mating readiness), or the enlargement of the penis and scrotum in the male and breasts in the female. We're going to stick with primary sex characteristics in this lesson, and this section is about the internal genitalia, what we call tubes.

During embryogenesis, both sexes have two sets of tubes: mesonephric and Müllerian ducts. The Müllerian duct's proper name is the paramesonephric duct, whereas the mesonephric duct has gone by the eponym of the Wolffian duct (Wolf for wolfman!). We made a judgment call about what we want to name these two ducts. Because the **mesonephric duct** is part of the mesonephros, which has a role in more than just the male internal genitalia, we are going to eschew the eponym in favor of calling it the mesonephric duct. But because "paramesonephric ducts" and "mesonephric ducts" sound so similar, and because so much literature refers to the Müllerian ducts and Müllerian-duct-related molecules (e.g., anti-Müllerian hormone), we are going to continue to use **Müllerian** ducts. From this point forward, no Wolffian ducts and no paramesonephric ducts. This also eliminates the temptation of using "m" as a shortcut—say mesonephric and Müllerian.

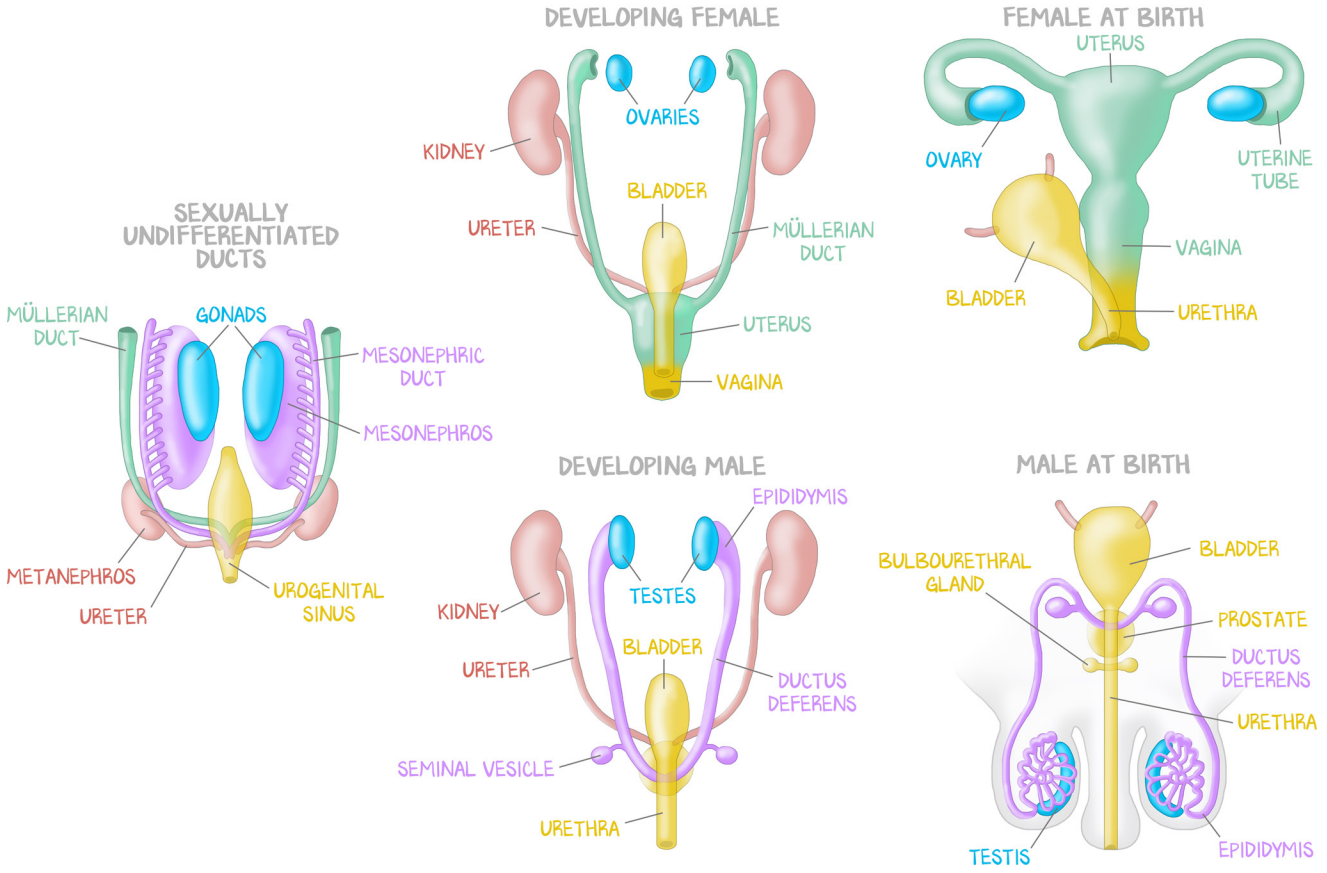

Figure 2.4: Destiny of the Tubes
In both sexes, the mesonephros will involute, and the kidneys will develop. In addition, the two pairs of ducts—the Müllerian ducts and the ducts of the mesonephros, the mesonephric ducts—either persist or involute while the gonad becomes either testis or ovary. In the female phenotype, the gonads become ovaries, the Müllerian ducts become the uterine tubes, uterus, and upper third of the vagina, and the mesonephric ducts involute. In the male phenotype, the gonads develop as testes, the Müllerian ducts involute, and the mesonephric ducts become the male tubes—epididymis, ductus deferens, and seminal vesicles.

The default phenotype is the female phenotype. If nothing happens, if there is no signal, the mesonephric duct involutes, and the Müllerian ducts form the uterine tubes, uterus, and upper third of the vagina. Two separate signals transform the female default to the male phenotype—involution of the Müllerian ducts and persistence of the mesonephric duct.

The **Sertoli cells** make **anti-Müllerian hormone** (AMH). AMH has also gone by the name Müllerian-inhibiting factor (MIF) and Müllerian-inhibiting substance (MIS), among others. We are only going to use AMH because anti-Müllerian hormone is very well named. The Müllerian ducts develop by default and are only induced to involution in the presence of AMH—AMH does not inhibit anything; rather, it stimulates apoptosis. In the presence of AMH, which means in the presence of Sertoli cells, the Müllerian ducts disappear entirely (except as the appendix of the testis).

Leydig cells make **testosterone**. Leydig cells lack 5α-reductase and, therefore, cannot make the more potent form of testosterone, 5**α**-dihydrotestosterone (DHT; see below). This justifies **testosterone** as the signal required for the mesonephric duct to persist. The presence of testosterone induces the mesonephric duct to become the ejaculatory duct, ductus deferens, seminal vesicles, and epididymis.

That testosterone is converted in peripheral tissues by **5α-reductase** into 5α-dihydrotestosterone, a many times more potent form of testosterone. It will transform the external genitalia from the default female phenotype to the male phenotype, discussed in the next section.

WHAT IT IS	WHERE IT COMES FROM	WHAT IT DOES
TDF protein	*SRY* gene on Y chromosome	Gonads become testes Support cells turn to Sertoli cells
Anti-Müllerian hormone	Sertoli cells	Involutes the Müllerian ducts
Testosterone	Leydig cells	Supports the mesonephric ducts; forms the ductus deferens, epididymis, seminal vesicle, and ejaculatory duct
5α-Dihydrotestosterone	5α-Reductase (not Leydig cells)	External genitalia become the male phenotype

Table 2.1: What Makes the Default Female Phenotype the Male Phenotype

External Genitalia Is Determined by 5α-Dihydrotestosterone

In both sexes, the development of the external genitalia begins through the same process, through the division of the cloaca into the urogenital sinus (which will become the bladder and how urine is eliminated) and the rectum (which will be how stool is eliminated), covered by the urogenital membrane. We saw this in Renal (urogenital sinus to bladder and urethra) and Gastrointestinal (formation of the rectum and colon). Now, we focus on how these structures relate to the external genitalia—in females, the lower two-thirds of the vagina, labia majora, labia minora, mons pubis, and clitoris; in males, the scrotum, penis, and glans of the penis. Like the primordial gonads, the primordial version of the adult structures is indifferent, capable of producing either the male or female phenotype (and *the default phenotype is the female phenotype*). Estrogen is *not* required for the female phenotype regarding the development of the external genitalia (this is an important distinction from secondary sex characteristics, which we will discuss in Female Reproduction, and an error committed by many educators and texts alike). **Testosterone is not enough** to masculinize the external genitalia. The enzyme **5α-reductase** converts testosterone to DHT (as we saw in prostate pathology in the Renal module). It is this potent form of testosterone that induces masculinization and the development of the male phenotype. These paragraphs are illustrated in Figure 2.5.

The **labioscrotal swellings** (aka the genital swellings, but because "labioscrotal" contains the two options of what the swellings can become—labia majora or scrotum—we opt for its use) surround the **genital fold** (genital swellings and genital folds are too easily conflated, another benefit of using labioscrotal swellings). The genital fold envelops the **urogenital membrane** (aka the cloacal membrane), and the genital fold is capped superiorly/cranially by a **genital tubercle**.

As the rectum and anus separate from the urogenital sinus, the external genitalia enter the late indifferent stage. Here, if DHT is present, these structures will adopt the male phenotype. If not, they will adopt the female phenotype.

The **genital tubercle** will become the glans in both sexes. It will become the glans (the most distal end) of the penis or the glans of the clitoris, the most anterior of which appears externally on visual inspection. The penis has a corpus cavernosum on each side. This is the erectile tissue that enables participation in the act of sex. In addition, the penis has a corpus spongiosum, through which the urethra runs. The clitoris, like the penis, is not just the glans of the clitoris. The clitoris has two corpora cavernosa that engorge with blood during sexual arousal. The crura (plural of crus, "leg") of the penis insert in the same location that the crura of the clitoris insert. The visualization we find most effective is to imagine the penis is formed by drawing the glans clitoris out from the body, the corpora cavernosa dragged into the external position, held in place by the crura.

The **labioscrotal swellings** will become either the scrotum in males or the labia majora and mons pubis in females. In the male phenotype, the products of the labioscrotal swellings, the scrota, grow down, hanging below the emergence of the penis, and eventually receive the testes. The two labioscrotal swellings fuse at the midline, denoted by the scrotal raphe. In the female phenotype, the labioscrotal swellings will become the labia majora as well as grow up to cover the clitoris and form the mons pubis.

The **genital folds** become the shaft of the penis, the corpus spongiosum, which contains the penile urethra in males. Like the labioscrotal swellings, the two genital folds will fuse, forming the penile raphe. In males, the urethra is "zipped up" from the base of the penis forward, extending from where the female urethra would have stopped, up through the shaft of the penis to the corona, where there is a transition from the genital-fold-derived shaft of the penis to the genital-tubercle-derived glans. To complete the urethral passage to the atmosphere (or amniotic fluid in utero), the ectoderm-derived glans of the penis must invaginate to meet the endoderm-derived urethra. In females, the genital folds do not fuse and become the labia minora. Because they do not fuse, the urogenital sinus brings the vestibule of the vagina and urethra close together.

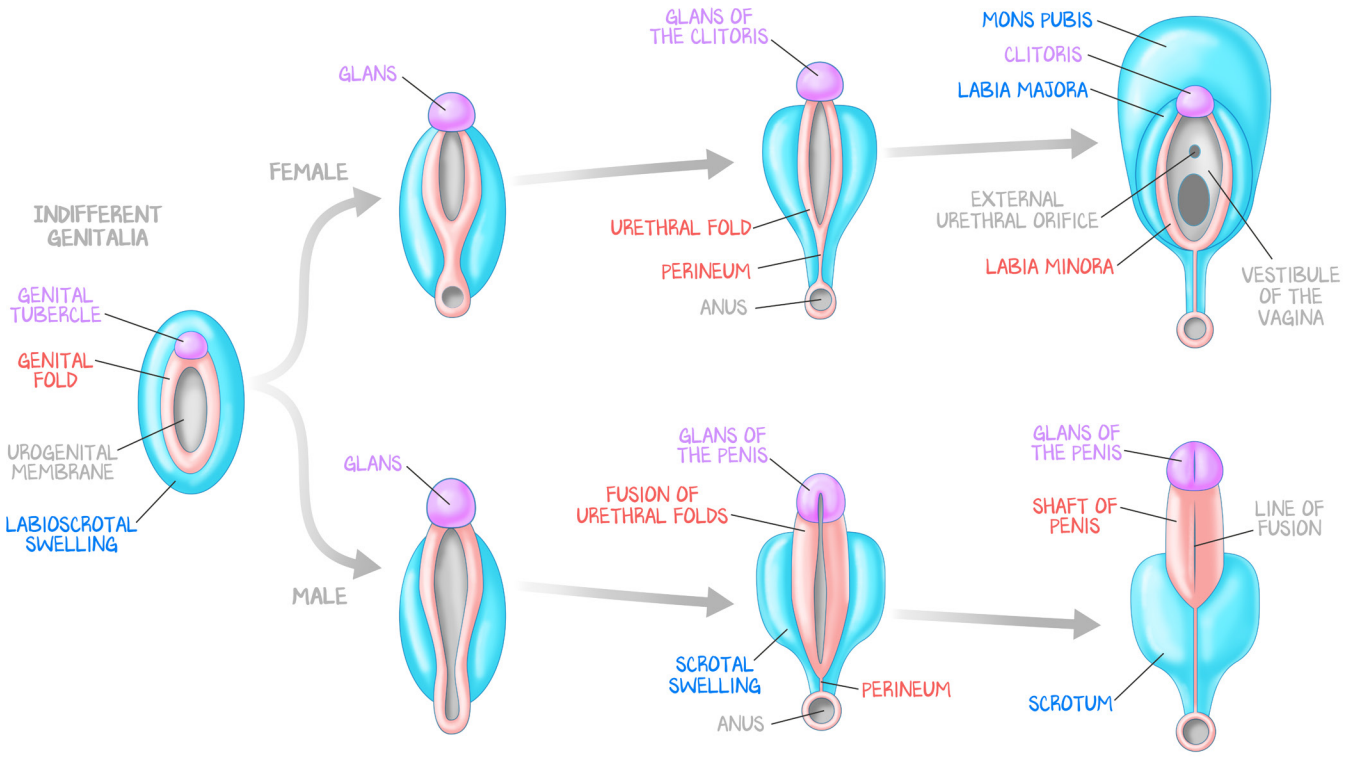

Figure 2.5: External Genitalia Formation
The cloaca, covered by a urogenital membrane, becomes the external genitalia of both sexes. The genital tubercle becomes the glans of either the penis or the clitoris. The labioscrotal swelling becomes the labia majora (which cover the labia minora and the internalized "shaft" of the clitoris) and mons pubis in females and the scrotum in males. The genital fold becomes the shaft of the penis, with its corpora cavernosa (erectile tissue) and corpus spongiosum (houses the urethra) in males, whereas it becomes the "shaft" of the clitoris, with its corpora cavernosa (erectile tissue) in females. However, this structure does not house the nearby urethra in females.

Testosterone is not strong enough to induce a change in external genitalia. Pathologic excess of adrenal androgens is enough to initiate the male phenotype, even with a female genotype (XX). We saw that in Endocrine: Adrenal #4: *Adrenal Hyperplasia NOS*.

The Urogenital Sinus, Urogenital Opening, and Hypospadias

In females, the fusion of the two Müllerian ducts forms the primordial uterus and upper vagina. How the lower two-thirds of the vagina develop is still debated, though it is likely to derive from the uretovaginal plate. The presence of Müllerian ducts are not required for the lower two-thirds of the vagina to form, as evidenced by pathologic conditions in which the uterus does not form. A signal other than that of the Müllerian ducts induces the ureterovaginal plate to bud off the **vaginal plate**, a solid cylinder of cells. The vaginal plate meets the Müllerian-derived upper vagina. The ureter buds off the structure that will become the lower vagina. Not surprisingly, then, that urethra exits near the exit to the vagina. Because the vagina grew within/behind the urogenital membrane, a remnant of that membrane, called the hymen, may persist (see Dr Williams' rant in Female Reproduction #4: *Cervix and Vagina*). In males, a small bud forms from the same part of the urogenital sinus that the vaginal plate does. In the male phenotype, that bud forms the **prostate and bulbourethral glands**.

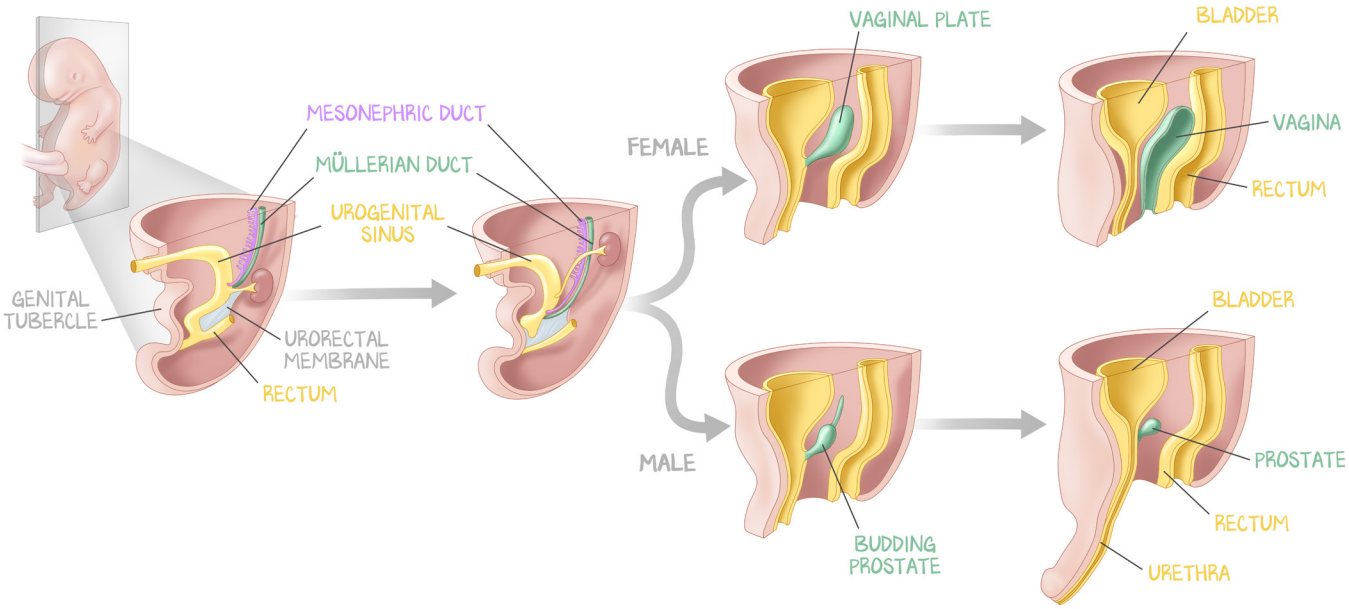

Figure 2.6: Vaginal vs. Prostatic Development from the Urogenital Sinus

In the female phenotype, the labia minora do not fuse, so there are no developmental disorders of the urethra at the urethral opening. In the male phenotype, there are no Müllerian ducts to fuse, and the opening that would have been the vagina closes as the labioscrotal swellings fuse to form the scrotum and the genital folds fuse to form the penis. That fusion of the swellings and folds is necessary to form the normal penis. The genital folds not only fuse but also proliferate to elongate the shaft of the penis. As they grow outward, they progressively fuse from the base of the penis towards the distal end. Failure to zip will result in a urethral opening located not at the tip of the glans but on the **ventral side** of the shaft, termed **hypospadias**. Hypospadias is the result of an embryological fusion defect of either the scrotal swellings or the genital folds. Because the spongy urethra is in the ventral penis, a defect in penile embryogenesis can result only in hypospadias—the urethral opening on the ventral side.

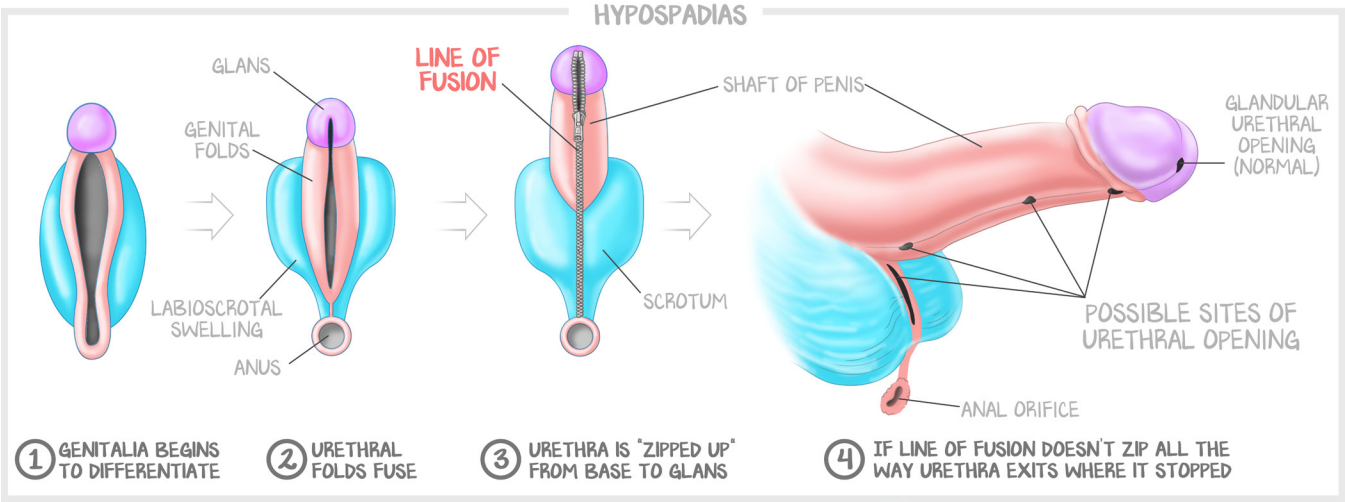

Figure 2.7: Disorders of Ventral Fusion—Hypospadias
The labioscrotal swellings become either the labia majora or the scrotum. The labioscrotal folds fuse at the midline to become the scrotum. The urethra extends from the female phenotype position out into the ventral aspect of the penile shaft. The genital folds, like the labioscrotal swellings, must fuse at the midline. They do so proximal to distal, as if zipping up the underside of the penis to include the urethra. Normal fusion results in the opening of the urethra on the top of the glans penis. Hypospadias is a failure of fusion or extension of the fusion (and thus the urethra), terminating on the underside of the penile shaft anywhere from the scrotum to the distal tip of the urethra.

Unrelated to hypospadias is the condition called **epispadias**. Epispadias is a urethral opening on the dorsal side of the penis. What is the dorsum of the penis continuous with? The dorsum of the penis is associated with the abdominal wall rather than the urogenital swellings and folds. Therefore, epispadias pathology is more akin to abdominal wall defects, such as gastroschises, omphalocele, and exstrophy of the bladder.

Disorders of Sexual Differentiation

Disorders of sexual differentiation (DSD) are conditions in which the genetic genotype is incongruent with phenotype. As we've seen, normal male phenotypic development requires the expression of the *SRY* gene and its gene product, TDF, to form the gonads, AMH to involute the Müllerian ducts, testosterone to sustain the mesonephric ducts, and DHT to masculinize the external genitalia. Normal XY development feeds forward—*SRY* leads to TDF, which leads to Sertoli cells, which lead to AMH and Leydig cells, which lead to testosterone, which leads to DHT—but multiple genes, receptors, and enzymes are required to move those processes forward. That is why we wanted you to think "gonads, tubes, external genitalia" in the beginning, because each of the three has a different signal.

Because *the default phenotype is the female phenotype*, the only DSD that can affect the XX genotype is the excess of something masculinizing that a girl shouldn't have. We discussed this in Endocrine: Adrenal #4: *Adrenal Hyperplasia NOS*—excess DHEAS due to excess ACTH released from the pituitary because of the adrenal glands' inability to produce cortisol produced ambiguous genitalia. The adrenal androgens produced in congenital adrenal hyperplasia (CAH) reach toxic levels far too late to affect the tubes or induce a normal male phenotype of the external genitalia, but early enough to affect the completion of the female external genitalia phenotype. So, the only XX DSD we want you to learn is CAH, in which the girl has female gonads (ovaries), female tubes (uterine tubes, uterus, upper third of vagina), but **ambiguous external genitalia**.

The remainder of the DSDs is XY DSD. If TDF is present, male gonads will develop. If AMH is present, the female tubes will involute. If testosterone is present, male tubes will develop. If DHT is present, male external genitalia will develop. Each of these scenarios is independent of the others and comes down to either the inability to secrete the signal or the inability to receive the signal.

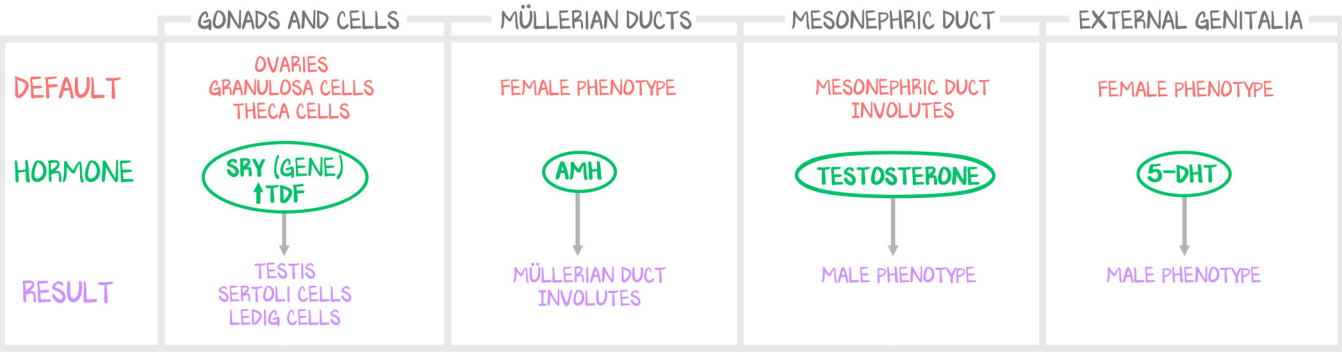

Figure 2.8: Disorders of Sexual Differentiation—Rules of the Game
The default phenotype is the female phenotype. If there is no *SRY* gene, there is no TDF, and the default is for the cortex to proliferate and the medulla to involute, so the gonad becomes the ovary. The cells will be oogonia/oocytes, granulosa cells, and theca cells. Without testosterone, the Müllerian ducts become the uterine ducts, uterus, and upper third of the vagina; the external genitalia become the clitoris, labia majora, and labia minora. If there is an *SRY* gene, then there is TDF, and the gonads become testes—the cortex involutes, and the medulla proliferates. The cells will be spermatogonia/spermatocytes, Sertoli cells, and Leydig cells. Independently, if anti-Müllerian hormone is present, the Müllerian ducts will involute. Independently, if the Leydig cells produce testosterone, the mesonephric duct will persist as the epididymis, ductus deferens, seminal vesicle, and ejaculatory duct. Both testosterone and 5α-reductase must be present to make DHT, which masculinizes the external genitalia—penis, glans penis, and scrotum.

In **5α-reductase deficiency**, the *SRY* gene is intact, so TDF is expressed, so the gonads are male. There is AMH, so the Müllerian tubes involute. There is testosterone, so the mesonephric ducts persist. However, 5α-reductase is required to transform testosterone to DHT. Without the enzyme, there is no DHT, so the external genitalia will develop with the female phenotype.

In **complete androgen insensitivity syndrome** (formerly testicular feminization), there is no testosterone receptor. This is a bit of an esoteric stretch because androgen insensitivity syndrome is rare on its own, but the hyperspecific complete androgen insensitivity syndrome is rare even among the androgen insensitivity syndromes. There are *varying degrees of insensitivity* in patients with androgen insensitivity, but that can't be how you are evaluated on this system. Because the licensure exams so clearly assess your knowledge of the system, they will likely only ever show you complete androgen insensitivity. In this condition, *SRY*/TDF, AMH, and testosterone are present. The only thing is that the testosterone signal cannot be heard by any cell. With *SRY*/TDF, there are male gonads, Sertoli cells, and Leydig cells. With AMH, the Müllerian ducts involute. Because the mesonephric ducts cannot hear the testosterone signal, they also involute—both sets of tubes involute. And because DHT is also an androgen, the external genitalia cannot hear the signal and, therefore, remain default female. This person develops as a normal-appearing girl and turns into a normal-appearing woman. All the testosterone of puberty will be converted into estrogen, which stimulates the development of secondary sex characteristics. And even though there is excess testosterone, without any receptor to act on, the high levels cause no apparent defect. This normal-appearing girl usually seeks medical attention because of normal (female phenotype) secondary sex characteristics without the onset of menses. Inspection on speculum exam demonstrates no cervix. An ultrasound reveals no uterus or ovarian tubes, and missing ovaries (because she has testes). The testes would not have descended into the scrotum (she doesn't have one) and will be found where the internal inguinal ring would have formed. Her karyotype will be XY. Undescended testes atrophy and can become malignant, so in addition to vaginal reconstruction (she has no proximal third of the vagina, so sex will be painful without vaginal elevation), the testes are removed after puberty.

In **gonadal dysgenesis** (which is extremely ultra-rare), there is a mutation in *SRY*. Because there is no *SRY* gene, the patient develops the default female phenotype, although she is genotypically XY. Unlike in complete androgen insensitivity, the process of forming the male phenotype never starts; thus, the default programming progresses forward. She is an entirely normal female with a Y chromosome.

	GONADS	FEMALE TUBES	MALE TUBES	GENITALIA
IF THERE IS . . .	***SRY*/TDF**	**AMH**	**TESTOSTERONE**	**DHT**
Normal (XX)	No = Ovaries	No = Persist Female phenotype	No = Involute Female phenotype	No = Female phenotype
Normal (XY)	Yes = Testes	Yes = Involute	Yes = Persist Male phenotype	Yes = Male phenotype
5α-Reductase deficiency (XY)	Yes = Testes	Yes = Involute	Yes = Persist Male phenotype	No = Female phenotype
Complete androgen insensitivity (XY)	Yes = Testes	Yes = Involute	No = Involute (no internal phenotype)	No = Female phenotype
Gonadal dysgenesis (XY) *"XY woman"*	No (mutation) = Ovaries	No = Persist Female phenotype	No = Involute	No = Female phenotype
Congenital adrenal hyperplasia (XY)	Yes = Testes	Yes = Involute	Yes = Persist Male phenotype	Yes = Male phenotype
Congenital adrenal hyperplasia (XX)	No = Ovaries	No = Persist Female phenotype	No = Involute	Yes = Masculinization of genitalia

Table 2.2: Disorders of Sexual Differentiation